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GENETIC VARIATION IN POPULATIONS OF THE HERMAPHRODITIC FLATWORM *MESOSTOMA LINGUA* (TURBELLARIA, RHABDOCOELA)

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ABSTRACT

Populations of the rhabdocoel *Mesostoma lingua* at a site in the Canadian arctic were polymorphic at two of ten allozyme loci. Phenotypic frequencies were determined in more than 1500 individuals from 35 populations at the polymorphic loci phosphoglucomutase and mannose-6-phosphate isomerase. Considerable gene frequency divergence was noted between populations only a few meters apart indicating that gene flow is low. Inbreeding coefficients due to population subdivision averaged 0.15, suggesting that existing populations receive an average of only one new migrant per generation. Genotypic frequencies in individual populations were ordinarily in good agreement with Hardy-Weinberg expectations, revealing that this hermaphroditic organism does not engage in self-fertilization.

INTRODUCTION

Simultaneous hermaphroditism is common in animal groups whose adult dispersal is limited either by a sessile life-style or by the occupation of a patchy habitat such as a pond or a host organism. Ghiselin (1969) has pointed out the obvious adaptive significance of self-fertilization in such organisms, particularly in habitat colonization. Laboratory studies have shown that many simultaneous hermaphrodites are capable of self-fertilization (Gee and Williams, 1965; Brenner, 1974). The few studies on natural populations of such hermaphrodites suggest that the incidence of self-fertilization can be high. Selander and Hudson (1976) showed that the gastropod *Rumina decollata* is ordinarily self-fertilizing and that natural populations consist of a number of genetically distinct, inbred lines comparable to those which exist in self-compatible plants (Jain, 1976). Similar observations have been made on a variety of hermaphroditic organisms including fish (Harrington and Kallman, 1968), anemones (Cain, 1974), and polychaetes (Beckitt, 1982).

The breeding systems of some major hermaphroditic groups, such as the turbellarian flatworms have been little studied. This class includes five orders of which the triclads and rhabdocoels are best known. Most triclads are obligate outcrossers, as isolated individuals fail to produce viable eggs (Hyman, 1951). However, members of the genus *Procerodes* are capable of self-fertilization and the freshwater triclad *Cura foremannii* is an obligate selfer (Anderson and Johann, 1958). Members of the rhabdocoel family Typhloplanidae typically produce two sorts of eggs—subitaneous and diapausing. The subitaneous eggs develop immediately and are thought to be self-fertilized, while the diapausing eggs are ordinarily thought to be cross-fertilized (Hyman, 1951). It is known, however, that some rhabdocoels are capable of producing resting eggs by self-fertilization (Sekera, 1906).

Although a large amount of work has been done on the cytogenetics of turbellarians (Bennazzi and Lentati, 1976) few studies of genetic variation in natural populations

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have been attempted. Biersma and Wijsman's (1981) survey of several European populations of the congeneric triclads *Polycelis nigra* and *P. tenuis* revealed variation at two enzyme loci. The patterns of variation did not, however, permit a simple genetic interpretation of their results. Other studies have examined the extent of genetic divergence among triclad taxa (Nixon and Taylor, 1976), but no studies of population structure have been attempted. The present study aimed to examine both the extent of inbreeding within populations and the amount of genetic divergence among local populations of the rhabdocoel flatworm, *Mesostoma lingua* (Abildgaard 1789). The genus *Mesostoma* includes more than 50 species (Ferguson and Hayes, 1941), and has a global distribution. Members of the genus typically produce both resting and subitaneous eggs. The subitaneous eggs are fragile, while the resting eggs are enclosed within a strong membrane. The former eggs initiate development immediately, while resting eggs are responsible for survival during periods of unfavorable climatic conditions. Members of the genus differ from most other turbellarians in being active predators on zooplankton as well as benthic invertebrates (Maly *et al.*, 1980; Schwartz and Hebert, 1982; MacIsaac and Hutchinson, 1985). *M. lingua* is broadly distributed through Eurasia (Ferguson and Hayes, 1941) and in the subarctic portions of North America (Holmquist, 1976). In Europe the species produces a series of generations via subitaneous egg formation (Heitkamp, 1977), but populations in northern Canada are univoltine (pers. obs.). Juveniles emerge from diapausing eggs in early June and are mature by mid–late July. Individuals produce a brood of resting eggs within the body and release them when they die in late August. The present study involved a survey of enzyme variability at two polymorphic loci in populations of *M. lingua* in pond habitats near Churchill, Manitoba.

Materials and Methods

Ponds were surveyed for *M. lingua* on two quartzite rock bluffs (A and C) located approximately 22 km east of Churchill, Manitoba. These bluff ponds have been the subject of detailed water chemistry and zooplankton distribution studies (Hebert and Billington, in prep.). These two bluffs are approximately 1.7 km apart, and have surface areas of 26,036 and 141,344 m^2 respectively. Some of the bluff ponds appear to sit within glacial scours, while others lie in fractures in the rock surface. The ponds on both bluffs had a similar mean surface area ($\approx$15 m^2) and a similar average depth ($\approx$0.4 m). The ponds show considerable variation in salinity, as a result of their varying proximity to Hudson Bay. The salinity variation reflects inputs of sea-spray as opposed to direct tidal flushing of the ponds. Many of the ponds are intermittent in years of low rainfall. Sixty ponds were present on Bluff A and 184 ponds on Bluff C.

The presence or absence of *Mesostoma lingua* in each pond was assessed by trapping during the period June 15–July 23, 1984. The traps were 100 ml jars whose lids were modified by the insertion of a 4 cm diameter piece of 1000 μm nylon mesh. Approximately 100 heat-killed zooplankton (*Diaptomus, Daphnia*) were placed inside each jar as bait. After baiting, the jars were placed on their side in the pond and marked with a float for easy recovery. Two traps were initially placed in each pond and left for a 24-hour period. If *Mesostoma* were not present when these traps were removed, the procedure was repeated on at least two occasions. In ponds containing *Mesostoma*, a single jar often trapped more than 100 animals, and both traps ordinarily contained animals.

Electrophoresis was carried out using cellulose acetate gels. Gels were presoaked for 30 minutes in pH 8.5 Tris Glycine (3 g Trizma, 14.4 gm Glycine in 1 l H_2O).

Mesostoma were macerated individually in sample wells and 12–24 samples applied to each gel plate. Gels were run for 15 minutes at 200 volts using a Tris Glycine buffer of the same molarity and pH as that used for soaking. Gels were stained using an agar overlay and standard staining solutions.

Five populations were screened for variation at ten loci including phosphoglucose isomerase, phosphoglucomutase (PGM), mannose phosphate isomerase (MPI), aldehyde dehydrogenase, lactate dehydrogenase, isocitrate dehydrogenase, 6-phosphogluconate dehydrogenase, glutamate oxaloacetate transaminase, amylase, and triose phosphate isomerase. Polymorphism was detected at only two loci—PGM and MPI. As MPI migrated more anodally than PGM it was possible to stain both enzymes on a single gel plate by using a stain mixture that contained the substrates and coupling agents for both enzymes. When possible, a sample of 48 individuals was electrophoresed for MPI and PGM from each of the ponds containing *Mesostoma*. These samples were collected between July 3 and 23, 1984, and included both late juvenile and adult individuals.

Electrophoretic analysis showed that recently fed *Mesostoma* contained active enzymes from their prey (Hebert *et al.*, in prep.). These enzymes were degraded within 2 days at 20°C, so collections of *Mesostoma* were generally held for several days at room temperature before electrophoresis.

Inbreeding coefficients were calculated using standard methods (Wright, 1978). $F_i = 1 - H_o/H_e$ where H_o is the observed heterozygosity and H_e is the amount expected at Hardy Weinberg equilibrium (H.W.E.). F_{is} was the weighted mean of the F_i values (*i.e.*, $F_{is} = \sum_{1}^{N} (n_i/n_t) F_i$ where n_i is the number of individuals sampled in a population, n_t is the total sample size; and N is the number of populations surveyed). The degree of divergence among populations was quantified using a hierarchical F analysis as described by Wright (1978). Three levels were recognized in the hierarchical analysis (demes, subdivisions, and total). The population of *Mesostoma* in an individual pond was treated as a deme, while the pooled populations on each of the two rock bluffs were treated as subdivisions of the total Churchill population. Based on gene frequency data, variances were calculated for gene frequencies among the populations in each subdivision (s^2_{DS}), and between subdivisions in the total (s^2_{ST}) and among demes in the total population (s^2_{DT}). Variances were corrected for sampling error as described in Wright (1978, pp. 86–89). The sampling variance for demes was, for instance, calculated using the formula $SE^2 = \frac{1}{N} \sum_{1}^{N} [q(1 - q)/n]$, where n is the number of genes sampled from a population, q is the frequency of the slow allele in a population, and N is the number of populations sampled. From the corrected variance estimates, inbreeding coefficients were calculated using the following formulae: $F_{DT} = s^2_{DT}/\bar{q}(1 - \bar{q})$; $F_{ST} = S^2_{ST}/\bar{q}(1 - \bar{q})$; and $F_{DS} = F_{DT} - F_{ST}/(1 - F_{ST})$ where $\bar{q}$ is the mean frequency of the slow allele in all populations. In the interpretation of these inbreeding coefficients it has been assumed that the allozyme variants at PGM and MPI have little or no effect on individual fitness.

Results

Distribution

M. lingua was the only large-bodied (>5 mm) rhabdocoel collected in the traps. Thirteen of the 60 ponds on Bluff A and 24 of the 184 ponds on Bluff C contained

M. lingua. Densities of the species in two (1, 38A) of the Bluff A ponds and in three (20, 29, 156) of the Bluff C ponds were too low to permit collection of the desired sample size for electrophoresis.

Allozyme variation

Twenty-four individuals from each of 3 ponds on Bluff A (4, 20, 60) and 2 ponds on Bluff C (13, 72) were examined for allozyme variation at 10 loci. Each population was monomorphic for the same allele at eight loci, but all were polymorphic at PGM and most were polymorphic at MPI. Phenotypic variation at both these loci could be explained by assuming the presence of two alleles. Homozygous phenotypes were single-banded and heterozygous phenotypes double banded, as expected on the basis of the quaternary structure of these enzymes.

Gene frequency variation among populations

Allozyme phenotypes at PGM and MPI were determined for more than 500 individuals from Bluff A and for more than 1000 individuals from Bluff C (Tables I, II). Twenty-four or more individuals were analyzed for each enzyme in 32 of the 35 populations investigated. Pronounced gene frequency variation was noted among these populations. Frequency of the slow allele of PGM varied among Bluff A populations from 0.25 to 0.76, and among Bluff C populations from 0.08 to 0.93. The variation in gene frequencies among Bluff A populations was highly significant ($G_{adj} = 80.57$, d.f. = 20, $P < 0.001$), as was that among Bluff C populations ($G_{adj} = 350.7$, d.f. = 40, $P < 0.001$). The mean frequency of the S allele of PGM on Bluff A did not differ from that on Bluff C ($G_{adj} = 0.94$, $P > 0.30$). Similar gene frequency variation was noted at MPI with the frequency of the slow allele varying from 0.46 to 0.94 on Bluff A and from 0.49 to 1.00 on Bluff C. The mean frequency of the S allele was significantly higher on Bluff C than on Bluff A ($G_{adj} = 77.9$, $P < 0.001$).

TABLE I

Genotypic frequencies at PGM and MPI for populations of M. lingua *on Bluff A*

	PGM Frequencies					MPI Frequencies				
	Genotypes			Gene		Genotypes			Gene	
Pond	SS	SF	FF	S	F_i	SS	SF	FF	S	F_i
A1	0	3	5	–	–	6	3	0	–	–
A3	15	21	12	.53	.12	38	10	0	.90	−.12
A4	10	23	14	.56	.01	38	10	0	.90	−.12
A5	28	17	3	.76	.03	9	26	13	.46	−.09
A6	10	23	15	.45	.03	15	20	13	.52	.17
A9	15	16	5	.64	.04	23	20	5	.69	.03
A20	4	22	22	.31	−.07	12	24	12	.50	0
A21	11	22	14	.47	.06	12	21	14	.48	.10
A38B	5	14	28	.25	.22	27	19	2	.76	−.09
A44	15	20	13	.52	.17	19	20	7	.63	.07
A45	11	25	11	.50	−.06	33	15	0	.89	−.19
A60	10	37	35	.35	−.005	51	7	0	.94	−.06
Total	134	243	177	.46		283	195	66	.70	

TABLE II

Genotypic frequencies at PGM and MPI for populations of M. lingua *on Bluff C*

	PGM Frequencies					MPI Frequencies				
	Genotypes			Gene		Genotypes			Gene	
Pond	SS	SF	FF	S	F_i	SS	SF	FF	S	F_i
C1	4	28	16	0.38	−.24	18	24	6	0.63	−.07
C13	13	37	24	0.43	−.02	73	1	0	0.99	−.001
C20	4	3	2	—		6	3	0	–	
C28	14	25	9	0.55	−.05	10	30	8	0.52	−.25
C29	3	5	7	–		6	4	5	–	
C37	9	29	10	0.49	−.21	12	23	13	0.49	.04
C40	10	27	11	0.49	−.13	39	9	0	0.91	−.10
C41	7	28	13	0.44	−.19	35	11	2	0.84	.13
C59	4	16	25	0.27	.09	31	11	1	0.85	.003
C60	18	24	6	0.63	−.07	39	8	1	0.90	.11
C61	6	25	17	0.39	−.10	37	11	0	0.89	−.13
C65	11	28	9	0.52	−.17	34	13	1	0.84	−.03
C66	38	8	2	0.88	.24	14	25	9	0.55	−.05
C70A	55	9	0	0.93	−.08	62	2	0	0.98	−.02
C72	9	31	12	0.47	−.20	52	0	0	1.00	
C83	0	6	30	0.08	−.09	21	3	0	0.94	−.07
C89	7	27	13	0.44	−.17	30	15	3	0.78	.08
C98	16	24	8	0.58	−.03	47	1	0	0.99	−.01
C100	4	17	18	0.32	−.001	26	6	0	0.99	−.10
C111	2	19	27	0.24	−.09	40	8	0	0.92	−.09
C134	13	21	14	0.49	.12	46	2	0	0.98	−.02
C134A	3	21	28	0.26	−.05	52	0	0	1.00	
C151	13	25	10	0.53	−.05	25	23	0	0.76	−.32
Total	263	483	311	0.48		755	234	49	0.84	

No microgeographical pattern was obvious in the gene frequency variation among populations on Bluffs A and C (Figs. 1, 2). Substantial gene frequency differences were, however, evident between populations only a few meters apart. Hierarchical F-analysis revealed that the pattern of gene frequency divergence was similar at the two loci (Table III). The large proportion of inbreeding was attributable to differences among populations on the same bluff, with at most a small contribution due to differences among bluffs. The mean inbreeding coefficient (F_{ST}) for the two loci was 0.151.

Genotypic frequencies in individual populations

Tests of genotypic frequencies at both the PGM and MPI loci in the 32 populations with large population sizes revealed only a single significant deviation from H.W.E., due to a heterozygote excess at the MPI locus in population C151. If there were a consistency in the direction of deviation from H.W.E., the mean of the F_i values (Tables I and II) would deviate significantly from zero. Analysis indicated however that these means (−0.03, PGM and −0.04 MPI) did not deviate significantly from zero ($t = -1.37$, $P > 0.10$ at PGM; $t = -2.02$, $P > 0.05$ at MPI). The F_{is} values corresponded closely with mean F_i values as sample sizes did not vary markedly among populations ($F_{is} = -.030$ for PGM, $F_{is} = -.036$ for MPI).

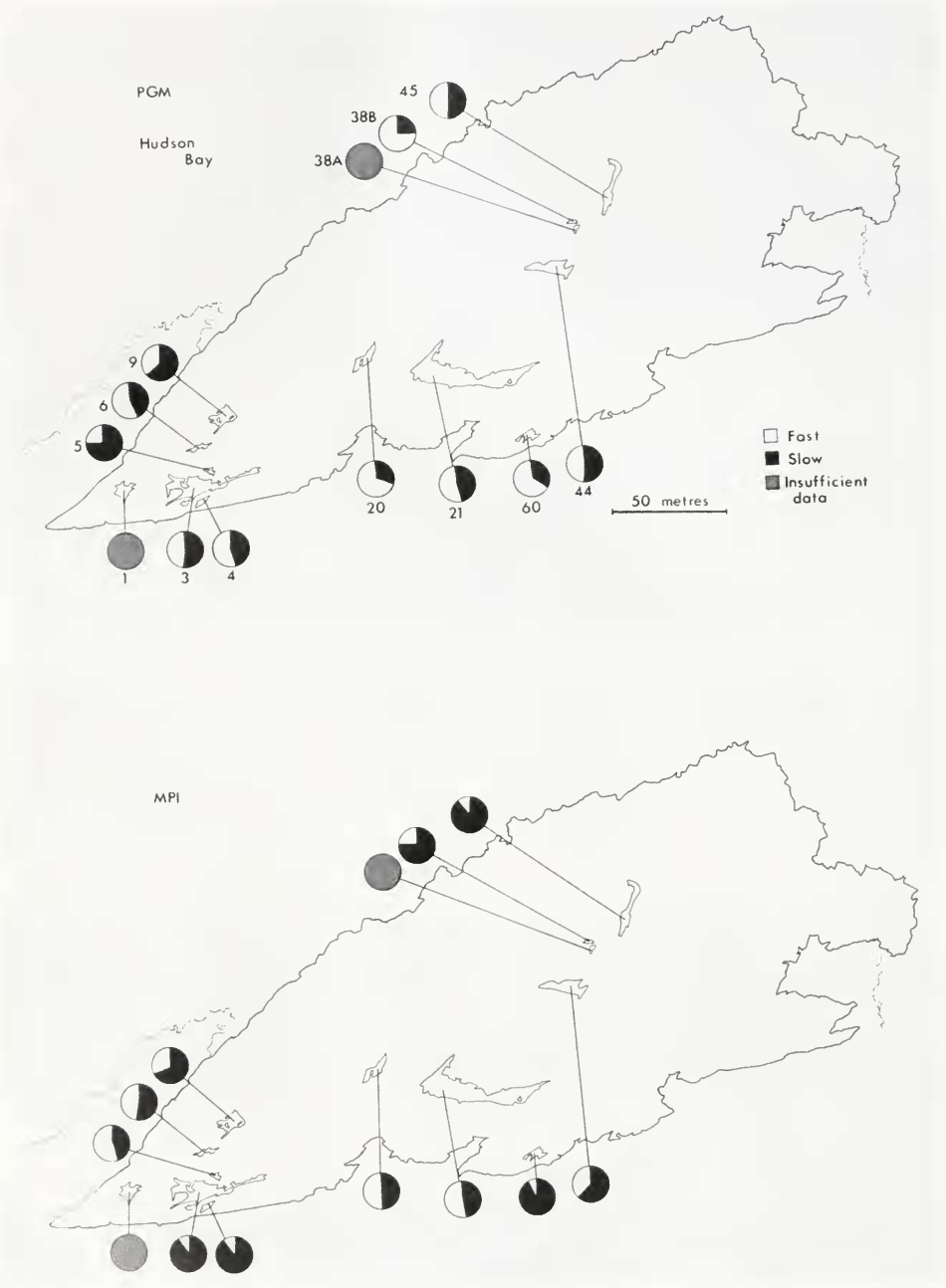

FIGURE 1. Gene frequency variation at PGM and MPI in populations of *Mesostoma lingua* on Bluff A.

Genotypic frequencies were determined for 9–15 individuals from three (A1, C20, C29) of the five low-density populations. These individuals represented the total population of *Mesostoma* in each habitat, as continued trapping failed to collect additional

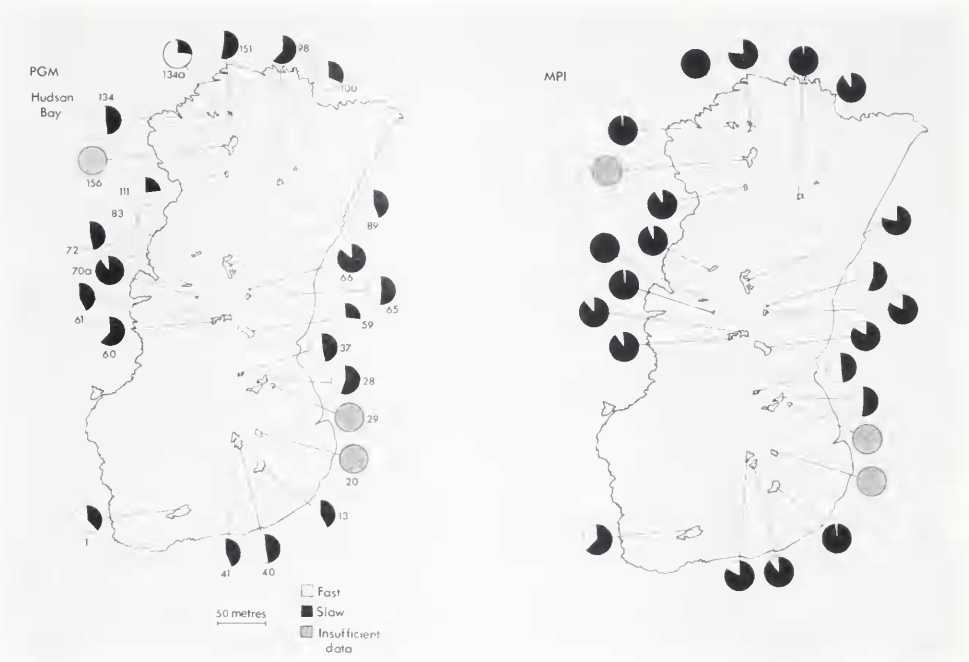

FIGURE 2. Gene frequency variation at PGM and MPI in populations of *Mesostoma lingua* on Bluff C.

specimens. None of these populations showed a significant deviation from H.W.E., although a heterozygote deficiency was evident at both MPI and PGM in the C29 population.

DISCUSSION

Mesostoma lingua is representative of a large number of aquatic invertebrates which rely on an unsophisticated means of dispersal. Not only are its diapausing eggs passively dispersed, but they lack structures to enhance dispersal such as those found in many terrestrial plants and some aquatic organisms (*e.g.*, ephippial cases of cladocerans). There is no direct information on the means by which *Mesostoma* is dispersed. The diapausing eggs remain within the adult's body until it dies, and during this period can be dispersed as a group. Shorebirds are probably an important dispersal

TABLE III

Differentiation of PGM and MPI frequencies among 32 populations of Mesostoma lingua *from 2 rock bluffs.*

	$\bar{q}$	ΣSE^2	Σs^2_{DS}	Σs^2_{ST}	Σs^2_{DT}	$\Sigma \bar{q}(1-\bar{q})$	F_{DS}	F_{ST}	F_{DT}
PGM	.47	.0045	.060	−.004	.0561	.498	.120	−.008	.113
MPI	.79	.0028	.057	.006	.062	.330	.175	.017	.189

$\bar{q}$ is the mean frequency of the slow allele, SE^2 is the sampling variance for demes, s^2 is the variance in gene frequencies and F is the inbreeding coefficient. D—deme, S—subdivision, T—Total. Summation signs indicate parameters produced by repeating and summing calculations for each allele (*i.e.*, for both the F and S allele in this case).

agent in intermittent ponds. As such ponds dry, the *Mesostoma* scavenge on the remains of large plankton (*e.g., Branchinecta paludosa*) and are likely to be ingested by birds which select the same food. In permanent ponds dispersal seems unlikely, except via overflow.

Like many other passively dispersed organisms, *Mesostoma lingua* has a broad range. Few studies have examined the amount of population differentiation in such organisms. Although it was long accepted (Mayr, 1963) that gene flow was extensive and local population differentiation minimal in such passively dispersed organisms, allozyme studies on populations of the cladocerans *Daphnia magna* (Hebert, 1974) and *Daphnia carinata* (Hebert and Moran, 1980) indicated marked gene frequency divergence among populations only a few kilometers apart. The generality of these results was questionable, as the parthenogenetic mode of reproduction of *Daphnia* permits populations to be founded from a single individual. However, the extent of local differentiation seen in *M. lingua* rivals that in the cladocerans and suggests that gene frequency divergence may be common among local populations of passively dispersed taxa.

Estimated population sizes of *M. lingua* in several Churchill ponds ranged from 1200–5000 individuals (Hebert, in prep.) indicating that drift is unlikely to be important in explaining the gene frequency differences among populations. Instead, it seems likely that most of the divergence is the result of populations being founded from few individuals. The population structure of *M. lingua* on individual rock bluffs conforms closely to Wright's (1978) island model. Populations are discrete and the probability of gene exchange between each may be similar because of the short distances involved. The mean F_{DS} of 0.15 suggests that populations receive only about 1.4 migrants per generation $\left(F_{DS} = \frac{1}{1 + 4\ N_e m}\right)$. As effective population sizes exceed 1000, migrants appear ordinarily to make up less than 0.1% of a population.

The observation that isolated individuals of *M. lingua* released diapausing eggs suggests that self-fertilization is possible (Hebert, in prep.). However, as attempts to hatch them failed, it was impossible to show that these eggs were viable. The close approach of genotypic frequencies in *M. lingua* populations to H.W.E. indicated that the eggs are rarely if ever self-fertilized in nature. Even if cross-fertilized, some inbreeding would be expected if population sizes were small. The genotypic data provide general evidence that most *M. lingua* populations are large enough so that this does not occur and agrees with the evidence from surveys of population size. It is premature to conclude that the breeding system of *M. lingua* is representative of that of turbellarians or even other rhabdocoels. Studies on the molluscan families Arionidae and Limacidae have shown considerable variation in reproductive behavior among taxa. Some species are facultative selfers, while others are either obligate selfers or outcrossers (McCracken and Selander, 1980; Foltz *et al.*, 1984). Closely related taxa tend to employ a similar breeding system, suggesting that a broad survey of species belonging to different turbellarian families would be the best way of assessing the extent of variation in breeding systems among these organisms.

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